

Diffusion limited reactions in confined environments

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We study the effect of confinement on diffusion limited bi-molecular reactions within a lattice model where a small number of reactants diffuse amongst a much larger number of inert particles. When the number of inert particles is held constant the rate of the reaction is slow for small reaction volumes due to limited mobility from crowding, and for large reaction volumes due to the reduced concentration of the reactants. The reaction rate proceeds fastest at an intermediate confinement corresponding to volume fraction near $1/2$ and $1/3$ in two and three dimensions, respectively. We generalize the model to off-lattice systems with hydrodynamic coupling and predict that the optimal reaction rate for mono-disperse colloidal systems occurs when the volume fraction is ~ 0.18 . Finally, we discuss the application of our model to bi-molecular reactions inside cells as well as the dynamics of confined polymers.

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It is a somewhat surprising fact that the total concentration of protein within a cell rivals that within a protein crystal[1]. This highly crowded environment plays an important role in dynamical processes, such as rates of chemical reactions, and thermodynamic properties, such as chemical equilibria, observed *in vivo*[2]. While the volume fraction of macromolecules within the cell may exceed 30%, there may be just a few copies of a given protein corresponding to a concentration of a few nanomolars for a cell of volume $1\mu\text{m}^3$ [4, 5]. This is in stark contrast to *in vitro* experiments where the reactants are present at a relatively high concentration with a negligible level of crowding molecules. Therefore, caution is required when interpreting *in vivo* biochemical experiments as it is not always obvious what effect the presence of crowding molecules will have. For example, the rate of a reaction may be increased if the crowding favors a compact transition state, decreased if the reaction is diffusion limited, or unaffected if the reactants are small compared to the crowding species[6].

In this letter we study the effect of crowding on reaction rates in a finite system with a fixed number of particles. A biological cell represents such a system. We find that the presence of non-reactive particles leads to a non-monotonic reaction rate as the volume of the system is changed. This non-monotonicity is the result of two distinct dynamical regimes. When the system volume is very large the effect of the crowding particles is negligible. Therefore, the rate of reaction is dictated by the time required for the reactants to diffuse the mean separation between reactive particles which increases with the system size. We will call such systems “concentration limited”. At the opposite extreme is the case where the system volume is very close to the sum of the total volume of the reactant and crowding particles. In this

case the separation between reactants may be quite small, but the reaction proceeds slowly because the high density of crowding particles severely impedes the diffusion of the reactants. Such systems are “crowding limited”. Between these two limits there is an optimal volume at which the reaction proceeds the fastest.

The competition between the concentration limited and crowding limited regimes may be understood through the following simple argument. The flux of particles at the surface of an absorbing sphere is $4\pi a c_r D$, where a is the radius of the sphere, and the concentration of particles far from the sphere, c_r , is inversely proportional to the volume of the system $c_r \propto R^{-3}$ [7]. The effect of crowding particles that do not react with the absorbing sphere may be included through a rescaling of the diffusion constant D provided the distance the reactant particles must travel is large compared to the mean spacing between crowding particles. For the case of a lattice-based system, a mean-field diffusion constant (which is exact when the crowding particles move much faster than the reactants) is achieved by multiplying the “bare” diffusion constant D_0 by the success rate for a particle move. This results in a diffusion constant of the form $D \sim D_0 p$ where $p = (1 - c)$ is the probability that the neighboring site for an attempted move is unoccupied, and c is the number density of particles on the lattice. Therefore, the flux at the absorbing surface scales as $a(1 - Nb^3 R^{-3})/R^3$, where N is the total number of particles in the system and b is the lattice constant. This expression has a maximum when the system size is such that the particle density is $1/3$.

In order to go beyond this simple argument, we have explored the transition from concentration limited reactions to crowding limited reactions using an analytically tractable model with two reactant particles that react instantaneously upon contact. With this simplification, the reaction “rate” is just the inverse mean first passage time for the particles to find each other. If we make the further simplification of holding one of the reactants

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fixed, the mean first passage time is given by

$$\bar{\tau} = \frac{1}{V} \int_V \tau(\vec{x}) d^d \vec{x} \quad (1)$$

where the integral is over the d -dimensional volume of the system. $\tau(\vec{x})$, the average time for the mobile reactant at \vec{x} to reach the stationary target, satisfies the equation [8]

$$D \nabla^2 \tau(\vec{x}) = -1. \quad (2)$$

Eq. 2 is subject to a reflecting boundary condition at the system periphery and an absorbing boundary condition at the surface of the stationary reactant.

If we place the fixed reactant at the center of a spherical box, as shown in Fig. 1, Eq. 1 is exactly solvable with the result (in two and three dimensions)

$$\begin{aligned} \bar{\tau}_{2D} &= \frac{1}{2D(c)(R^2 - a^2)} \left(R^4 \ln \left(\frac{R}{a} \right) - \frac{3R^4}{4} + R^2 a^2 - \frac{a^4}{4} \right) \\ \bar{\tau}_{3D} &= \frac{6}{D(c)(R^3 - a^3)} \left(\frac{R^6}{3a} - \frac{3R^5}{5} + \frac{R^3 a^2}{3} - \frac{a^5}{15} \right). \end{aligned} \quad (3) \quad (4)$$

Here a is the radius of the stationary target, and $D(c)$ is the concentration dependent diffusion constant. In the spirit of the mean-field argument introduced earlier, we employ a lattice model and utilize an excellent approximation for the self-diffusion of a lattice gas derived by van Beijeren and Kutner [10]. For reactant and crowding particles with equal mobilities on a square lattice, the self-diffusion constant takes the form

$$D(c) = \frac{\Gamma b^2}{8} \left(\sqrt{4(1-c) + c^2(\pi-1)^2} - c(\pi-1) \right), \quad (5)$$

where b is the lattice spacing, Γ is the attempt rate for particle moves, and c is the ratio between the total number of mobile particles to the number of accessible sites $c \simeq b^2(N+1)/(\pi(R^2 - a^2))$. From Eqs. 3 and 5 we find an expression for the reaction time which is plotted in Fig. 2.

We have performed Monte Carlo simulations of this model consisting of one reactant and N inert particles confined to a two dimensional circular box of radius R with a circular target of radius a placed at the center, as shown in Fig. 1. Each run of the simulation begins with a random configuration of the reactant and crowding particles and ends when the reactant reaches the center target at which point the first passage time is recorded. The average reaction time is determined from 10,000 such runs.

For the purposes of our simulation we define the box and the target to include all sites on the lattice with a distance to the origin less than or equal to R and a , respectively. Although the definition of a circular box on a square lattice is somewhat cumbersome, this geometry is advantageous relative to square or rectangular boxes because the circular box allows for non-integer adjustments of the linear box dimension with minimal change to the

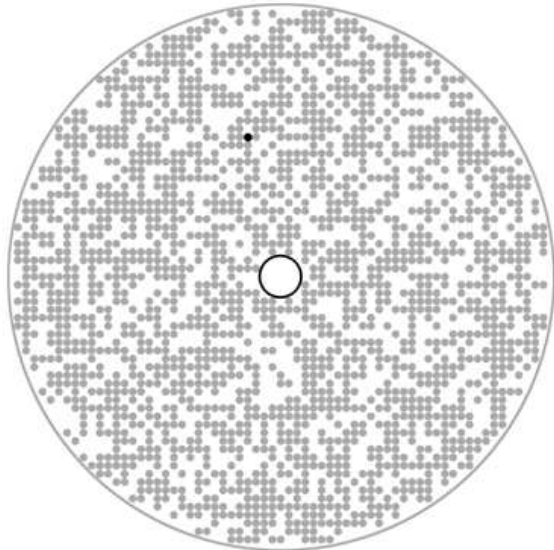


FIG. 1: Snapshot from the simulation described in the text. The stationary target is indicated by the black circle in the center, and the mobile reactant is the black dot halfway between the target and the top of the outer circle. Also shown are the 2000 inert crowding particles (grey).

overall geometry. This fine-tuning of the box size greatly increases the number of data points that can be collected in the crowding limited regime.

The simulation used $N = 2000$ crowding particles which, like the single mobile reactant, each occupied a single lattice site. The central target had a larger size $a = 3$ to minimize the discreteness effect of the lattice when comparing to the continuum theory given by Eq. 1. The radius of the confining box was varied from $R = 150$ to $R = 26.7$ lattice spacings, corresponding to densities from $c = 0.03$ to $c = 0.90$. The results are shown in Fig. 2.

The simulation results show a minimum in the reaction time very near the minimum of 36.9 predicted by Eq. 3 (corresponding to 47% of the sites being occupied). The reaction times diverge sharply when the box radius becomes less than 29 lattice sites, or the concentration exceeds $\sim 75\%$. At large box radii the reaction times increase as R^2 and approach the reaction rate in the absence of crowding particles (green line). As shown in Fig. 2 the difference between the crowded and uncrowded reaction times is nonzero at all system sizes. This can be explained by noting that while the probability that a given site is occupied scales as R^{-d} , the required number of steps scales like R^d . The result is that the number of time steps where the particle is unable to move due to crowding remains nearly constant as the system size is increased.

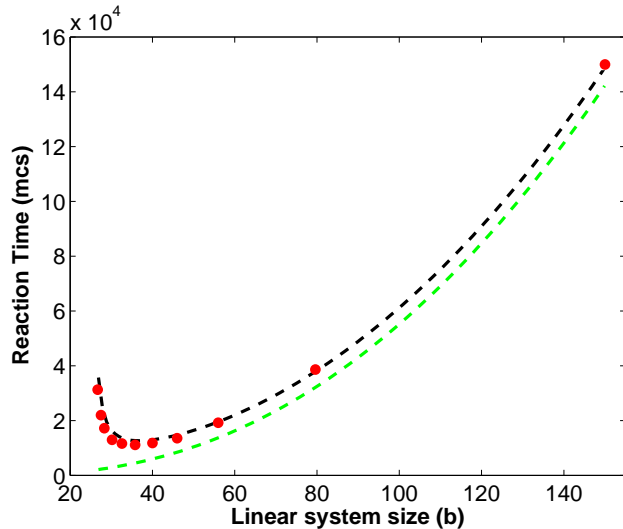


FIG. 2: (color online) Comparison of the reaction time predicted by Eq. 1 (black dashes) to the simulation data (red dots). The green dashes indicate the reaction time predicted in the absence of crowding particles. Error bars (not shown) are smaller than the size of the dots.

The reaction times in Fig. 2 deviate from the theoretical curve at system sizes below $50b$ with a maximum error of 39% at the highest concentration simulated. This discrepancy is due to a non-diffusive, logarithmic correction to the mean squared displacement (MSD) in the lattice gas system[11]. This correction allows the reactant to sample its immediate surroundings more efficiently than a purely diffusive particle. The effect of the correction term can be seen in Fig. 3 where we compare the MSD of the reactant in our simulations to the expected MSD (as calculated from the Green's function) for a random walker in a circle with diffusion constant given by Eq. 5. At the largest system size, when the particle density is 0.1, the agreement between the simulation and calculated MSD is better than 3% at all times. At $R = 35.8$ and $R = 26.7$, where the particle density is 0.5 and 0.1 respectively, the discrepancy between the observed and calculated MSD is 8% and 18% respectively at short times and shrinks at later times as the MSD saturates.

Our results can be extended to the more physically relevant three dimensional, off-lattice system with a few modifications. We recall that hard sphere systems undergo a glass transition at a volume fraction $\phi_c \sim 0.58$, which is less than the close packing density[9]. Although the system is not completely frozen, the relevant time scales diverge sharply. Therefore, we restrict our analysis to densities below ϕ_c . In the fluid regime, for $\phi < \phi_c$, the self diffusion constant is modified not only by the short range excluded volume interactions, but also by long range hydrodynamic coupling mediated by the solvent[19]. This latter effect is not included in

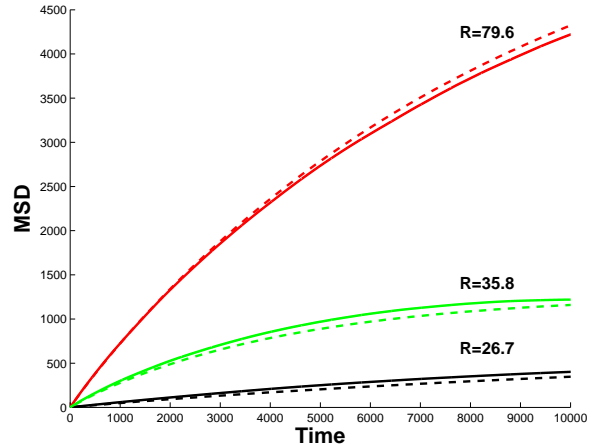


FIG. 3: (color online) The mean mean squared displacement of the mobile reactant measured in our simulation (solid lines) compared to the calculated mean squared displacement for a random walker in a circle(dashed lines).

our lattice model due to the lack of solvent. Using the self-diffusion constant derived in reference [19] (equation 4.34), together with Eq. 4, we predict that the optimal reaction time for mono-disperse, hard-core, Brownian spheres occurs when the system size is such that the volume fraction is ~ 0.18 .

Experimentally, a crowded reaction with adjustable volume could be realized using microfluidic techniques. In this case the tracer and crowding particles would be confined to a micro droplet reaction vessel whose volume could be controlled through osmotic gradients across a semi-permeable barrier[12]. This system mimics *in vivo* experiments that show increased tracer particle diffusion in osmotically swollen cells[13, 14].

In most systems of interest the target reactant would be mobile rather than stationary. One expects that the ratio of the reaction time for the stationary target to the reaction time for two mobile reactants in a fixed box size should be independent of the number of crowding particles present, as the crowding particles merely rescale the diffusion time. Our simulations support this intuition, and we find that the ratio is ~ 1.6 and ~ 2.0 in two and three dimensions respectively, with corrections on the order of a/R .

In the context of these theoretical results it is natural to ask to what extent do cells optimize biochemical reaction rates by adjusting their size? At first glance it would seem unlikely that cell volume would be a useful parameter for the cell to use to regulate reaction rates due to the large number of reactions that occur simultaneously. Furthermore, differential protein expression rates during the cell cycle could modify the overall protein concentration and thereby alter the cytoplasmic dif-

fusion rate[15]. However, it is well known that the cell is more complicated than a “bag of enzymes,” and therefore, the potential exists for the cell to compartmentalize reactions in such a way that the volumes of the compartments are individually tunable. For example, the digestion of a pathogen within a macrophage occurs within a vesicle created by endocytosis. The volume of this vesicle is, in principal, adjustable by the amount of membrane used during vesicle creation. Similarly, other membrane-bound organelles such as golgi, endoplasmic reticulum, mitochondria, and the nucleus could be individually adjusted to optimize reactions occurring within. This is consistent with the finding that diffusion rates in the mitochondria and endoplasmic reticulum can differ substantially from the cytoplasm[16, 17].

The case of the cell nucleus deserves special consideration. Here the primary reactions of importance involve the manipulation of the genome which is encoded by DNA. In analogy to our two reactant model, many of the genome management functions the cell performs require two specific portions of the DNA to find each other. These internal cyclization reactions may occur between monomers separated by polymer spacers ranging from less than a persistence length, up to lengths on the order of the chromosome size[20, 21]. If we identify the polymer segments that flank and bridge the reacting monomers as crowding particles, then we can immediately generalize the preceding argument for the rate of crowded reactions to the internal cyclization of a confined polymer. Specifically, the rate of internal cyclization will have a non-monotonic dependence on the size of the box containing the polymer. This non-monotonicity has been previously

observed in computer simulations[22].

The onset of crowding limited dynamics at small system sizes leads to non-monotonic behavior in the relaxation of other structural properties of the polymer such as the end-to-end vector[23]. This non-monotonicity is quantitatively different from that of crowded reactions due to the sub-diffusive behavior of the monomers imposed by the connectivity constraint. However, it can be easily explained by noting that the initial effect of reducing the volume accessible to the polymer to smaller than its unconfined size is to reduce the conformational phase space that the polymer can sample. This allows the polymer to sample the phase space faster resulting in shorter relaxation times. However, like the free particle case, as the monomer density approaches the close-packing limit the polymer becomes jammed and the relaxation times lengthen.

We have shown that a simple, analytically tractable model is able to quantitatively predict reaction times occurring within crowded environments. This model can be generalized to off-lattice systems and systems with explicit solvent with slight modification. The non-monotonicity in the reaction times shown here has broad implications for reactions within cells as well as the dynamics of confined polymers.

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